Clinical Cancer Research

A Randomized Phase II/III Study of Naptumomab Estafenatox + IFNα versus IFNα in Renal Cell Carcinoma: Final Analysis with Baseline Biomarker Subgroup and Trend Analysis

Robert E. Hawkins[1], Martin Gore[2], Yaroslav Shparyk[3], Vladimir Bondar[4], Oleg Gladkov[5], Tosho Ganev[6], Mihai Harza[7], Serhii Polenkov[8], Igor Bondarenko[9], Petr Karlov[10], Oleg Karyakin[11], Rustem Khasanov[12], Gunnar Hedlund[13], Goran Forsberg[13], Örjan Nordle[13], and Timothy Eisen[14]

Abstract

Purpose: To prospectively determine the efficacy of naptumomab estafenatox (Nap) + IFNα versus IFN in metastatic renal cell carcinoma (RCC).

Experimental Design: In a randomized, open-label, multicenter, phase II/III study, 513 patients with RCC received Nap (15 μg/kg i. v. in three cycles of four once-daily injections) + IFN (9 MU s. c. three times weekly), or the same regimen of IFN monotherapy. The primary endpoint was overall survival (OS).

Results: This phase II/III study did not meet its primary endpoint. Median OS/PFS for Nap + IFN patients was 17.1/5.8 months versus 17.5/5.8 months for the patients receiving IFN alone ($P = 0.56$; HR, 1.08/$P = 0.41$; HR, 0.92). *Post hoc* exploratory subgroup and trend analysis revealed that the baseline plasma concentrations of anti-SEA/E-120 (anti-Nap antibodies) for drug exposure and IL6 for immune status could be used as predictive biomarkers. A subgroup of patients (SG; $n = 130$) having concentrations below median of anti-SEA/E-120 and IL6 benefitted greatly from the addition of Nap. In SG, median OS/PFS for the patients treated with Nap + IFN was 63.3/13.7 months versus 31.1/5.8 months for the patients receiving IFN alone ($P = 0.02$; HR, 0.59/$P = 0.02$; HR, 0.62). Addition of Nap to IFN showed predicted and transient immune related AEs and the treatment had an acceptable safety profile.

Conclusions: The study did not meet its primary endpoint. Nap + IFN has an acceptable safety profile, and results from *post hoc* subgroup analyses showed that the treatment might improve OS/PFS in a baseline biomarker-defined RCC patient subgroup. The results warrant further studies with Nap in this subgroup. *Clin Cancer Res; 22(13); 3172–81. ©2016 AACR.*

Introduction

Renal cell carcinoma (RCC) accounts for 2% to 3% of all new cancer cases (1). Clear cell RCC is the most common subtype and accounts for 70% to 80% of all RCC. Development of new therapies, for example, tyrosine kinase inhibitors (TKI), has improved the median survival of patients with advanced RCC to about 26 months.

Improvement of therapy of advanced RCC by introducing new concepts is still urgent even though there have been major advancements including the establishment of the TKIs and mTOR inhibitors as first- and second-line RCC treatments lately (1, 2). Immunotherapy is well on the way to becoming an established tool in the cancer treatment armory and RCC is regarded as a sensitive tumor type. Cytokines and especially high-dose IL2 showed activity as immune therapy of RCC but the toxicity and limited efficacy has restricted its application (3). Recently mAbs targeting the programmed death-1 (PD-1) pathway have shown great promise also in the treatment of advanced RCC (4, 5). Anti-PD-1 and anti-CTL-associated antigen 4 (anti-CTLA-4) denominated checkpoint modulators are examples of mAbs blocking immunosuppressive pathways often active in cancer patients (6, 7). Immune therapy of cancer has developed tremendously and now this group of therapies contains a wide spectrum of immune activation approaches including direct immune stimulation as well as blocking of immune suppression (8). To achieve the ultimate goal of cure in cancer patients, it might be necessary to carefully and selectively combine treatments with distinct mechanisms of action including also both checkpoint

[1]The Christie NHS Foundation Trust, Manchester, United Kingdom. [2]Royal Marsden Hospital NHS Trust, London, United Kingdom. [3]State Regional Treatment and Diagnostics Oncology Center, Lviv, Ukraine. [4]Public Clinical Treatment and Prophylaxis Institution, Donetsk, Ukraine. [5]Chelyabinsk Regional Clinical Oncology Dispensary, Chelyabinsk, Russia. [6]Urology Clinic General Hospital for Active Treatment "St. Anna", Varna, Bulgaria. [7]Fundeni Clinical Institute, Bucharest, Romania. [8]Public Treatment and Prophylaxis Institution, Chernihiv Regional Oncology Center, Chernihiv, Ukraine. [9]Municipal Institution Dnipropetrov, Dnipropetrovsk, Ukraine. [10]City Clinical Oncology Dispensary, St. Petersburg, Russia. [11]Medical Radiological Research Center, Obninsk, Russia. [12]Republic Oncology Dispensary, Kazan, Russia. [13]Active Biotech AB, Lund, Sweden. [14]Cambridge University Health Partners, Addenbrooke's Hospital, Cambridge, United Kingdom.

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Corresponding Author: Robert E. Hawkins, Christie CRC Research Centre, Manchester, M20 4BX, United Kingdom. Phone: +44 16 1446 3208; Fax: +44 16 1446 3299; E-mail: Robert.Hawkins@ics.manchester.ac.uk

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Translational Relevance

Cancer patients must have no/low immune suppression to fully benefit from T-cell–stimulating immunotherapy. In this phase II/III trial with Naptumomab estafenatox (Nap), a 5T4-selective immunostimulating tumor therapeutic protein, a *post hoc* analysis subgroup of patients with advanced renal cell carcinoma (RCC) having baseline concentrations below median of drug-binding antibodies and IL6 (no/low immune suppression) benefitted greatly from addition of Nap to treatment with IFNα. In the subgroup, median OS/PFS for the patients treated with Nap + IFNα was 63.3/13.7 months versus 31.1/5.8 months for the patients receiving IFNα alone ($P = 0.02$; HR, 0.59/$P = 0.02$; HR, 0.62). Our data showed that Nap has promising antitumor activity in the subgroup of patients with RCC with good drug exposure and without overexpression of the inflammatory cytokine IL6. Furthermore, as baseline IL6 appears to be prognostic and predictive of outcome on treatment with TKIs and immunotherapies, this may be a stratification factor for future RCC studies.

modulators and direct and selective immune stimulators. Here, we present complete phase II/III results with a tumor-selective immune stimulator, Naptumomab estafenatox/ABR-217620/ANYARA (Nap), which easily can be combined with, for example, checkpoint modulators as well as with TKIs.

Antibody targeting of super antigens to tumor cells combines powerful T-cell cytotoxic activity with a targeted approach to eradicate tumor cells. Tumor-targeted super antigens are recombinant fusion proteins that consist of an antitumor Fab moiety genetically fused to a super antigen. Nap contains the 5T4 antibody and the SEA/E-120 super antigen (9, 10). 5T4 recognizes an antigen expressed on a large number of solid tumor forms including RCC with an affinity in the order of 1 nmol/L (9, 10). Nap induces T-cell–mediated killing of tumor cells at concentrations around 10 pmol/L and the super antigen moiety has been engineered to have low binding to human antibodies and MHC class II (9, 10). After phase I studies (11), a prospective, randomized phase II/III trial was conducted in RCC (12, 13). Patients were randomized 1:1 in an open-label study to receive Nap + IFNα or IFN. Nap (15 μg/kg) was given intravenously in three cycles of four once-daily injections plus IFN (9 MU s.c. three times weekly) or the same dose and schedule of IFN mono therapy. The primary endpoint was OS. Secondary endpoints were PFS, response rate, and safety. Here we present the final results and a baseline biomarker subgroup and trend analysis.

Materials and Methods

Patients

This was a multinational, multicenter, randomized, open-label, parallel-group, phase II/III study in patients with confirmed metastatic or inoperable locally advanced RCC eligible for standard therapy with IFN. Additional key eligibility criteria were histologically or cytologically confirmed clear cell or papillary type RCC, Karnofsky performance status $\geq$ 70, favorable or moderate risk group prognosis by Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY) risk score criteria (score 0–2), life expectancy > 3 months, and acceptable levels of specific hematology and serum chemistry parameters. This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice, and was approved by each center's Regulatory and Ethics Committees. All participants provided written informed consent.

Study design

After screening and enrollment, patients with RCC were randomized 1:1 to receive Nap + IFN or established treatment with IFN. Stratification was to establish balance between the treatment arms with regard to a prognostic index based on MSKCC risk and first- versus second-line of treatments. Patients in the active arm 1 were given 15 μg/kg Nap i.v. in three cycles of four once-daily injections plus IFN, 9 MU s.c. three times weekly, except for the Nap treatment weeks or the same dose, and schedule of IFN monotherapy. Nap treatment cycles were given at weeks 1, 9, and 17 and IFN during all other weeks up to 18 months, and IFN monotherapy in treatment arm 2 was given from week 1 up to 18 months with the option in both arms to continue beyond this if the patient was benefiting.

Endpoints and assessments

The primary endpoint was OS. Secondary endpoints were PFS, response rate, immunologic response to treatment in patients receiving Nap, pharmacokinetics, and safety. The main analysis on OS data from all patients was predefined to be executed at expected 383 events. For PFS, results underwent radiographic assessment in accordance with RECIST. Safety and tolerability were assessed throughout the study by physical/clinical examination, hematology and biochemistry tests, and monitoring AEs, which were graded according to the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0.

Biomarkers and immunopharmacology

Multiple blood samples were collected at specific time points (baseline and during treatment) for anti-SEA/E-120 and cytokines. Cytokines (IL2, IL6, IL10, IFNγ, and TNFα) as a measure for immune activation were assayed at 0 and 3 hours on days 1 and 2 of the Nap treatment cycles. Baseline plasma IL6 and all anti-SEA/E-120 concentrations were measured with ELISA methods and the plasma cytokine response patterns were analyzed with a cytometric bead array.

Statistical methods and analysis

OS and PFS were evaluated with the log-rank test stratified by MSKCC risk. The other stratification factor, first- or second-line treatment, was not used due to too few patients in the second line. Cox regression was used for analyses of baseline covariates including treatment versus covariate interactions. Preplanned [intention-to-treat (ITT) population] and exploratory multivariate analyses were conducted on baseline prognostic factors. All analyses were done with SAS (SAS Institute Inc.).

Treatment versus covariate interaction analysis was done with the objective to explore whether any prognostic features identify patients with RCC more likely to benefit from Nap treatment. Preplanned (stratification factors, clear versus non-clear RCC, liver metastasis vs. non-liver metastasis, biomarkers, geographical area, gender, nephrectomy versus non-nephrectomy and exposure, cytokines, anti-SEA/E-120 in arm 1 only) and exploratory subsets were analyzed using Kaplan–Meier/Cox methodology. Cox models were used, one for each baseline factor, with treatment and baseline as main effects

and the treatment-by-baseline factor interaction to test whether the treatment effect differed for patients with varying levels of the specific factor. A three-way *post hoc* interaction analysis was conducted on baseline anti-SEA/E-120, IL6, and treatment. Statistics for exploratory analyses are provided for descriptive purposes only, as the primary endpoint was not met. The exploratory analyses were not adjusted for multiple comparisons.

To further describe the influence of covariates, explorative moving median and adapted subpopulation treatment effect pattern plot (STEPP; ref. 14) analyses were performed. The ITT population was divided into eight groups based on anti-SEA/E-120 with overlap resulting in subgroups of at most 40% of the patients to describe the potential differences in efficacy between treatment arms due to baseline anti-SAE/E-120 (Supplementary Fig. S1) and IL6 levels.

Results

Patients

From May 2007 to October 2010, 521 patients with RCC were randomized 1:1 in an open-label study to receive Nap + IFN or IFN. Patients with clear cell or papillary RCC received either Nap (15 μg/kg given i.v. in three cycles of four once-daily injections) plus IFN (9 MU s.c. three times weekly) or the same dose and schedule of IFN monotherapy except for weeks with Nap treatment. Five hundred thirteen patients in Bulgaria (76), Romania (56), Russia (188), Ukraine (153), and the United Kingdom (40) were treated (ITT) with a median follow-up time for censored patients of 43 months. The great majority were white patients receiving first-line treatment for clear cell RCC. Baseline characteristics were well balanced and are summarized in Table 1.

Efficacy

OS. A total of 373 deaths (73% of patients) had occurred at the predefined final OS analysis in the ITT population. Median OS for the patients treated with Nap + IFN was 17.1 months versus 17.5 months for the patients receiving IFN alone ($P = 0.56$; HR, 1.08; Fig. 1A). No difference of OS between treatment arms in the ITT population was detected, and accordingly, the study did not reach its primary endpoint. Interestingly, and as discussed below, a *post hoc* exploratory subgroup of patients was defined having prolonged OS after addition of Nap (Figs. 1B and C).

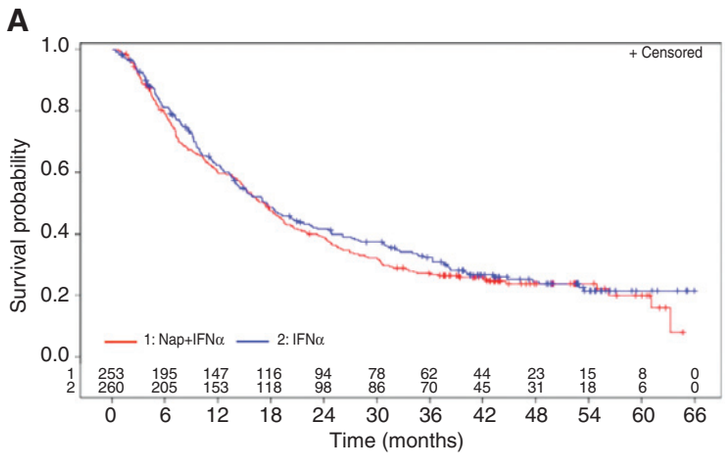
PFS. A total of 452 patients had progressed or had died (88% of patients) at the final PFS analysis in the ITT population. Median PFS for the patients treated with Nap + IFN was 5.8 months versus 5.8 months for the patients receiving IFN alone ($P = 0.41$; HR, 0.92; Fig. 2A). No difference of PFS between treatment arms in the ITT population was detected. In accordance with OS and as discussed below, a *post hoc* exploratory subgroup of patients showed prolonged PFS after addition of Nap (Figs. 2B and C).

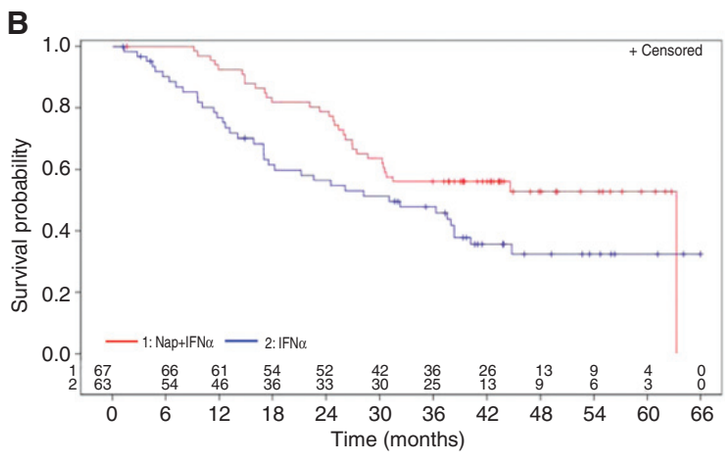
Overall tumor response. The best overall tumor response results were similar in the two treatment arms with 6 complete responses (CR) and 29 partial responses (PR) for the patients treated with Nap +IFN and 4 CR and 36 PR for the patients receiving IFN alone.

Exploratory subgroup analysis. Phase I studies with Nap showed low baseline plasma anti-SEA/E-120 antibody levels in most patients and indicated that the exposure of clinically relevant doses of Nap are independent of baseline antibodies (11). In this study, the number of patients was vastly expanded and the geographic inclusion changed. In certain territories, increased levels of baseline anti-SEA/E-120 and thus low Nap exposure were unexpectedly detected (Supplementary Table S1). Patients in the United Kingdom had expected levels of anti-SEA/E-120 and Nap exposure. The analysis of Nap concentration in plasma showed decreased drug levels with increasing anti-SEA/E-120. Spearman rank correlation analysis showed a statistically significant ($P < 0.0001$) inverse relationship between baseline anti-SEA/E-120 antibody concentration and plasma concentration of Nap in the ITT population at 1 hour of the first day of the first treatment cycle (Supplementary Fig. S2 illustrates that patients having increased anti-SEA/E-120 had decreased Nap exposure; trend analysis with identical overlapping patient subsets also used below for IL2 response, OS, and PFS). The ITT subgroup having below median of baseline anti-SEA/E-120 showed a tendency to a beneficial OS (Fig. 1C) and PFS (Fig. 2C).

Table 1. Demographic and baseline characteristics of the ITT and SG populations

		ITT		SG	
		Nap + IFNα (*n* = 253)	**IFNα (*n* = 260)**	**Nap + IFNα (*n* = 67)**	**IFNα (*n* = 63)**
Age, median (range)		58 (25–79)	57 (19–83)	60 (28–78)	57 (26–83)
Sex, *n* (%)	Females	70 (28)	77 (30)	20 (30)	23 (37)
	Males	183 (72)	183 (70)	47 (70)	40 (64)
Ethnic origin, *n* (%)	White	253 (100)	258 (99)	67 (100)	62 (98)
	Asian	0 (0)	2 (1)	0 (0)	1 (2)
ECOG performance status, *n* (%)	0	164 (65)	159 (61)	50 (75)	45 (71)
	1	89 (35)	100 (39)	17 (25)	18 (29)
MSKCC risk subgroup, *n* (%)	Favorable	152 (60)	152 (59)	38 (57)	32 (51)
	Intermediate	101 (40)	108 (42)	29 (43)	31 (49)
Line of treatment, *n* (%)	1st	248 (98)	250 (96)	65 (97)	59 (93)
	2nd	5 (2)	10 (4)	2 (3)	4 (6)
Histopathologic type, *n* (%)	Clear cell carcinoma	237 (94)	238 (92)	62 (93)	59 (94)
	Papillary cell carcinoma	13 (5)	18 (7)	5 (8)	4 (6)
	Other	3 (1)	4 (2)	0 (0)	0 (0)
Baseline biomarkers, median	Anti-SEA/E-120 pmol/mL	53	54	34	36
	IL6 pg/mL	6.7	7.2	3.3	3.2

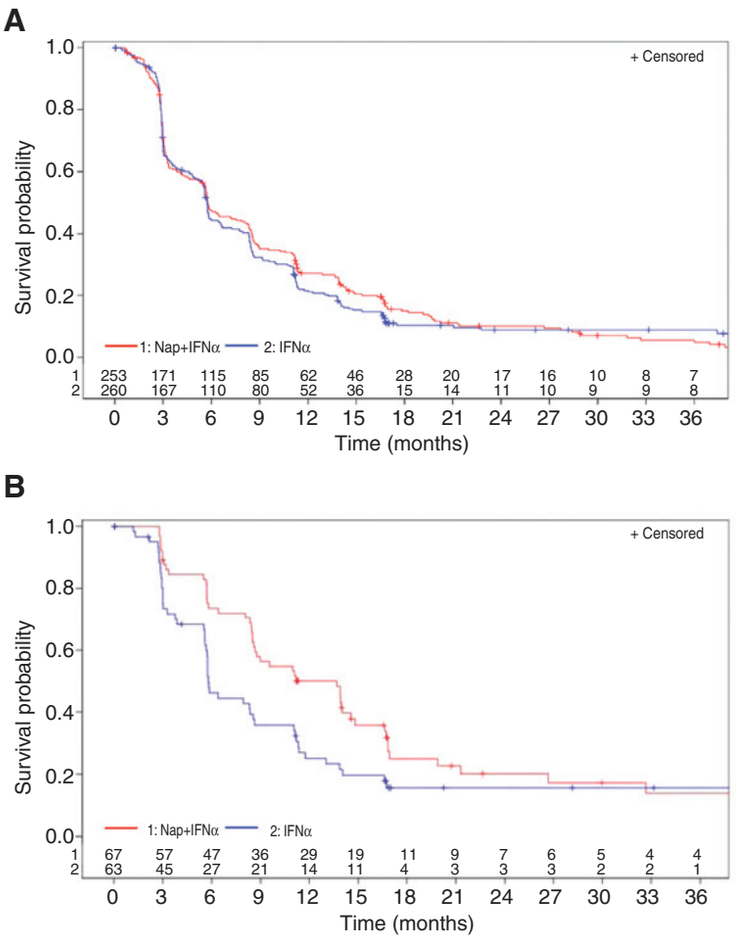




C

Subgroup	Level	Patients	Events	Medians	HR with 95% CI
ITT		253 | 260	189 | 184	17.1 | 17.5	1.08 (0.88–1.33)
MSKCC	Good	101 | 108	68 | 74	24.3 | 23.2	0.96 (0.69–1.34)
	Intermediate	152 | 152	121 | 110	14.9 | 13.5	1.13 (0.87–1.46)
HENG	Good	33 | 41	18 | 27	26.9 | 27.9	0.74 (0.41–1.35)
	Intermediate	188 | 171	144 | 121	17.8 | 18.5	1.16 (0.91–1.47)
	Poor	32 | 48	27 | 36	6.6 | 9.2	1.28 (0.77–2.11)
Karnofsky	70	9 | 9	8 | 7	12.0 | 9.2	1.01 (0.36–2.85)
	80-100	244 | 251	181 | 177	17.4 | 17.6	1.08 (0.88–1.33)
Platelets	High	28 | 42	26 | 33	6.7 | 9.6	1.26 (0.75–2.11)
	Normal	225 | 218	163 | 151	18.5 | 20.2	1.10 (0.88–1.38)
Neutrophils	High	29 | 26	22 | 21	8.5 | 11.1	1.17 (0.64–2.12)
	Normal	224 | 234	167 | 163	17.9 | 18.4	1.07 (0.86–1.33)
LDH	High	15 | 4	13 | 2	10.8 | 9.1	1.53 (0.34–6.84)
	Normal	238 | 256	176 | 182	17.8 | 17.5	1.04 (0.85–1.28)
Hemoglobin	Low	87 | 100	70 | 76	12.9 | 9.9	0.97 (0.70–1.35)
	Normal	166 | 160	119 | 108	19.6 | 24.8	1.14 (0.88–1.48)
Calcium	High	40 | 47	29 | 31	17.3 | 14.4	1.10 (0.66–1.84)
	Normal	213 | 213	160 | 153	17.1 | 18.5	1.08 (0.86–1.34)
Nephrectomy	Yes	206 | 209	148 | 146	17.9 | 17.1	1.03 (0.82–1.30)
	No	47 | 51	41 | 38	14.9 | 18.4	1.37 (0.88–2.13)
Diagnosis	CCC	237 | 238	175 | 168	17.3 | 17.5	1.07 (0.87–1.33)
	PCC	13 | 18	11 | 13	15.3 | 13.5	1.03 (0.46–2.31)
Line	First	248 | 250	185 | 177	17.3 | 17.6	1.09 (0.89–1.34)
	Second	5 | 10	4 | 7	14.1 | 12.5	0.94 (0.27–3.23)
Region	CEE	235 | 238	178 | 164	16.3 | 18.2	1.16 (0.94–1.43)
	UK	18 | 22	11 | 20	27.4 | 12.4	0.48 (0.23–1.02)
Anti-SEA/E-120	>median	121 | 117	100 | 80	12.0 | 14.4	1.41 (1.05–1.89)
	≤median	126 | 115	85 | 84	23.2 | 17.8	0.85 (0.63–1.15)
IL6	>median	121 | 119	107 | 97	7.5 | 12.5	1.40 (1.06–1.85)
	≤median	126 | 113	78 | 67	27.9 | 31.4	0.96 (0.70–1.34)
IL6 or anti-SEA/E-120	>median	180 | 169	154 | 126	10.8 | 14.2	1.38 (1.09–1.75)
IL6 and anti-SEA/E-120	≤median	67 | 63	31 | 38	63.2 | 31.0	0.59 (0.37–0.95)

Figure 1.
Overall survival depicted by Kaplan–Meier curves (A and B) and Forest plot (C) of ITT (A), the SG (B), and subgroups based on baseline criteria (C).



C

Subgroup		Patients	Events	Medians	HR with 95% CI
ITT		253 | 260	224 | 228	5.7 | 5.7	0.92 (0.77–1.11)
MSKCC	Good	101 | 108	90 | 98	8.3 | 5.7	0.80 (0.60–1.07)
	Intermediate	152 | 152	134 | 130	5.3 | 5.7	1.02 (0.80–1.30)
HENG	Good	33 | 41	30 | 36	11.2 | 8.4	0.72 (0.44–1.18)
	Intermediate	188 | 171	165 | 151	5.7 | 5.7	0.94 (0.75–1.18)
	Poor	32 | 48	29 | 41	3.2 | 3.0	1.13 (0.70–1.82)
Karnofsky	70	9 | 9	9 | 8	3.0 | 6.4	1.52 (0.56–4.13)
	80-100	244 | 251	215 | 220	5.8 | 5.7	0.90 (0.74–1.09)
Platelets	High	28 | 42	28 | 38	3.0 | 5.0	1.12 (0.68–1.84)
	Normal	225 | 218	196 | 190	6.4 | 5.7	0.92 (0.75–1.13)
Neutrophils	High	29 | 26	25 | 22	3.1 | 5.0	1.17 (0.65–2.08)
	Normal	224 | 234	199 | 206	5.9 | 5.7	0.91 (0.75–1.10)
LDH	High	15 | 4	14 | 4	2.9 | 2.8	1.00 (0.32–3.12)
	Normal	238 | 256	210 | 224	5.9 | 5.7	0.89 (0.74–1.07)
Hemoglobin	Low	87 | 100	79 | 85	5.6 | 5.5	0.99 (0.73–1.34)
	Normal	166 | 160	145 | 143	5.9 | 5.7	0.89 (0.70–1.12)
Calcium	High	40 | 47	31 | 37	4.5 | 6.0	1.15 (0.71–1.86)
	Normal	213 | 213	193 | 191	5.8 | 5.6	0.89 (0.72–1.08)
Nephrectomy	Yes	206 | 209	181 | 183	5.7 | 5.7	0.94 (0.76–1.15)
	No	47 | 51	43 | 45	7.5 | 5.8	0.78 (0.51–1.21)
Diagnosis	CCC	237 | 238	209 | 207	5.8 | 5.7	0.93 (0.77–1.13)
	PCC	13 | 18	12 | 17	5.5 | 3.0	0.76 (0.35–1.63)
Line	First	248 | 250	220 | 218	5.7 | 5.7	0.93 (0.77–1.13)
	Second	5 | 10	4 | 10	2.7 | 5.6	0.60 (0.16–2.28)
Region	CEE	235 | 238	208 | 208	5.7 | 5.7	0.93 (0.77–1.13)
	UK	18 | 22	16 | 20	8.0 | 3.9	0.65 (0.33–1.29)
Anti-SEA/E-120	>median	121 | 117	111 | 103	4.3 | 5.7	1.08 (0.82–1.41)
	≤median	126 | 115	108 | 99	7.5 | 5.7	0.81 (0.62–1.07)
IL6	>median	121 | 119	113 | 109	3.3 | 5.7	1.23 (0.95–1.61)
	≤median	126 | 113	106 | 93	9.5 | 5.8	0.77 (0.58–1.02)
IL6 or anti-SEA/E-120	>median	180 | 169	168 | 152	4.1 | 5.7	1.11 (0.89–1.38)
IL6 and anti-SEA/E-120	≤median	67 | 63	51 | 50	13.7 | 5.8	0.62 (0.42–0.92)

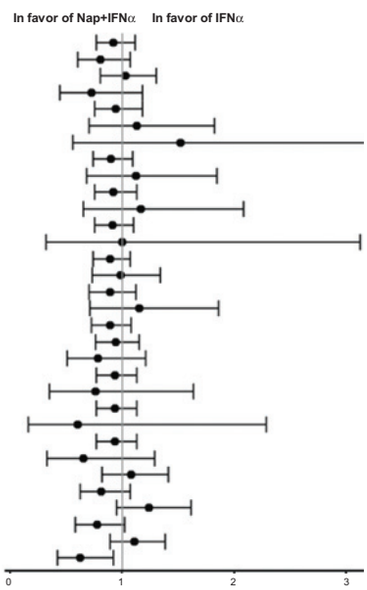


Figure 2.
Progression-free survival depicted by Kaplan–Meier curves (A and B) and Forest plot (C) of ITT (A), the subgroup (SG; B), and subgroups based on baseline criteria (C).

Table 2. Univariate and multivariate Cox regression analyses on OS for ITT patients; all patients and separate treatment arms

	Univariate			Multivariate	
Covariate	**Change in Akaike Information Criteria**	***P***	**HR with 95% CIs**	***P***	**HR with 95% CIs**
ITT population ($n = 513$)					
Age <median	−2.0	0.95	1.01 (0.82–1.23)		
Calcium risk	−2.0	0.94	1.01 (0.77–1.33)		
Nap + IFNα	−1.4	0.45	1.08 (0.88–1.33)	0.035	1.26 (1.02–1.56)
Karnofsky < 80	−1.0	0.29	1.32 (0.79–2.22)		
Male	−0.8	0.26	0.88 (0.70–1.10)		
Not nephrectomized	0.8	0.088	1.24 (0.97–1.59)		
Anti-SEA/E-120 ≤median	2.8	0.029	0.79 (0.64–0.98)		
LDH Risk	3.6	0.0091	1.99 (1.19–3.34)		
Neutrophils risk	5.5	0.0037	1.61 (1.17–2.21)		
Weight ≤median	6.9	0.0029	1.36 (1.11–1.67)		
Number of MSKCC risks	22.0	0.00000067	1.41 (1.23–1.62)		
Hemoglobin risk	22.9	0.00000031	1.73 (1.40–2.13)		
Karnofsky score	31.0	<0.00000001	0.96 (0.95–0.98)	0.000017[a]	0.97 (0.96–0.98)
Number of heng risks	38.7	<0.00000001	1.41 (1.27–1.57)	0.00014[a]	1.25 (1.11–1.39)
IL6 ≤median	75.6	<0.00000001	0.38 (0.31–0.47)	<0.00000001[a]	0.44 (0.35–0.56)
ARM: Nap + IFNα ($n = 253$)					
Anti-SEA/E-120 ≤median	7.5	0.0021	0.63 (0.48–0.85)	0.026	0.72 (0.54–0.96)
IL6 <median	58.0	<0.00000001	0.31 (0.23–0.41)	<.000000001	0.32 (0.24–0.43)
ARM: IFNα ($n = 260$)					
Anti-SEA/E-120 ≤median	−2.0	0.92	1.02 (0.75–1.38)	0.48	1.12 (0.82–1.52)
IL6 ≤median	21.3	0.0000018	0.47 (0.34–0.64)	0.0000013	0.46 (0.33–0.63)

[a]Backward elimination of covariates with $P < 0.001$ was kept in the model.

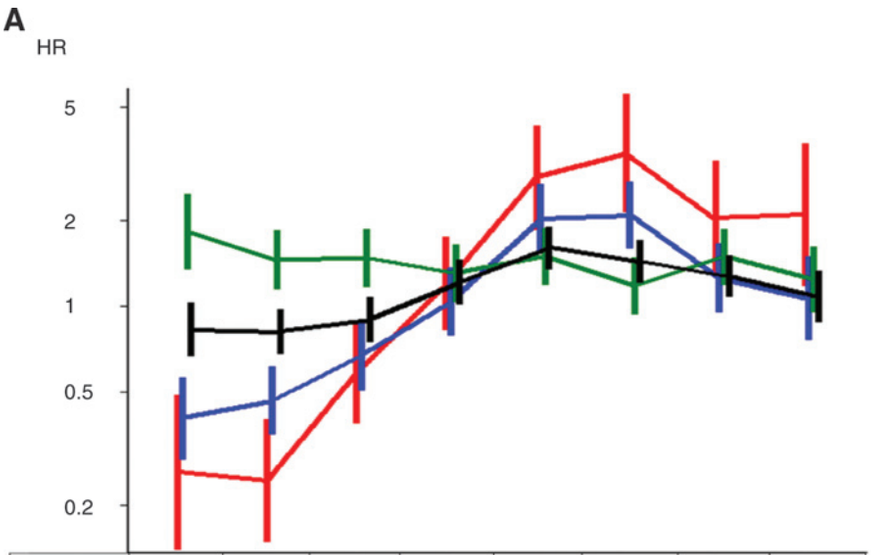
The ITT population and the subgroup having below median of baseline anti-SEA/E-120 were analyzed in uni- and multivariate analysis of parameters important for OS (Table 2). The most important parameter in both ITT (Table 2) and the low baseline anti-SEA/E-120 subgroup (data not shown) was baseline plasma concentration of IL6 in both the uni- and multivariate analysis. The prognostic MSKCC and Heng risk scores as well as their individual prognostic factors were less important as compared with IL6. IL6 was shown to be prognostic for OS for patients on the IFN alone arm (Table 2) in accordance with previous studies (15). Furthermore, when comparing the two treatment arms in ITT, a trend of greater importance of IL6 for OS in arm 1, the Nap arm, was shown (Table 2). Baseline anti-SEA/E-120 was prognostic for OS in the Nap arm while it had no prognostic value for patients treated with only IFN (Table 2). The Forest plots indicate a tendency for better antitumor effects in the Nap arm in patients with less advanced disease having lower risk scores (Figs. 1C and 2C). Using baseline anti-SEA/E-120, correlating with exposure, and the selected prognostic factor IL6 in a three-way test for interaction showed statistical significant interaction (Supplementary Table S2) indicating different antitumor activity in different patient subsets. Furthermore, when analyzing interaction of different prognostic features including IL6 and anti-SEA/E-120 for exposure, these two used together emerged as clearly significant with an HR of 2.315, the ratio between OS HRs for patients having IL6 or anti-SEA/E-120 >median versus IL6 and anti-SEA/E-120 ≤median (Supplementary Table S3). The selected baseline plasma biomarkers were therefore used and the patients were analyzed for treatment effects in the subgroup (SG; $n = 130$) treatment having below median of anti-SEA/E-120 and IL6 thereby excluding patients that might not benefit from Nap. A total of 69 deaths (53% of patients) had occurred at the final OS analysis in SG. Median OS for the patients treated with Nap + IFN was 63.3 months versus 31.1 months for the patients receiving IFN alone ($P = 0.02$; HR, 0.59; Fig. 1B and C). A total of 101 patients had progressed or had died (78% of patients) at the final PFS analysis in SG. Median PFS for the patients treated with Nap + IFN was 13.7 months versus 5.8 months for the patients receiving IFN alone ($P = 0.02$, hazard ratio 0.62; Fig. 2B and C).

In SG ($n = 130$) the best overall tumor response was higher in the Nap-treated patients ($n = 67$) with 3 CR and 16 PR for the patients treated with Nap + IFN and no CR and 10 PR for the patients receiving IFN alone.

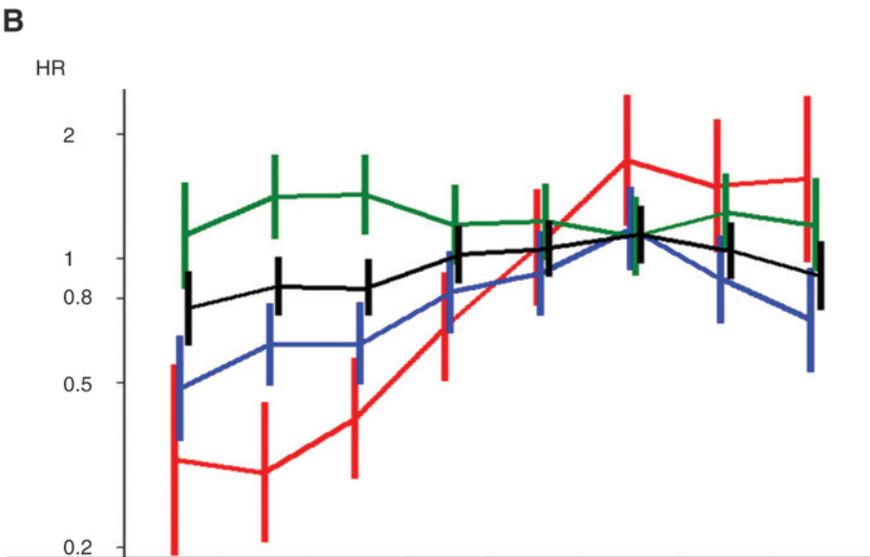
Trend analysis of OS and PFS. To further analyze the relation between baseline anti-SEA/E-120 and IL6 on antitumor effect parameters, a trend analysis was performed. The STEPP analysis shows clear trends of improving OS and PFS (HRs shown in Fig. 3A and B) in patients with decreasing anti-SEA/E-120 and IL6. The patients with anti-SEA/E-120 <36.7 pmol/mL and IL6 <3.24 pg/mL display HRs of 0.26 and 0.32 for OS and PFS, respectively. The patients defined by the three lowest anti-SEA/E-120 blocks having IL6 below median display HRs ≤0.68 and ≤0.62 for OS and PFS, respectively. Patients with high anti-SEA/E-120 or IL6 have HRs >1, most pronounced at high anti-SEA/E-120 and low IL6.

Immunopharmacology

Antibodies binding to Nap (anti-SEA/E-120). Nap is a fusion protein of bacterial and mouse origin. Baseline levels of antibodies binding to SEA/E-120 are detectable in all patients with a median of 53 pmol/mL in this study. The anti-SEA/E-120 titers increased after Nap treatment to a median of nearly 18,000 pmol/mL at week 9 after 1 cycle of Nap and a median above 13,000 pmol/mL at week 25 after the third cycle (Supplementary Fig. S3). For the SG population, the antibody titers were increased but to a lower degree.



Anti-S	13.8–36.7	13.8–43.2	28.2–53.9	35.4–67	41.8–84.2	51.7–125	65.2–4705	81.5–4705
IL6	1	2	3	4	5	6	7	8
<3.24 (red)	0.26(26)	0.25(43)	0.59(50)	1.21(57)	2.84(51)	3.45(41)	2.04(36)	2.1(22)
<7.02 (blue)	0.41(73)	0.47(104)	0.68(102)	1.04(100)	2.03(92)	2.09(91)	1.26(81)	1.07(54)
>7.02 (green)	1.83(53)	1.46(82)	1.48(89)	1.31(91)	1.5(99)	1.18(100)	1.5(105)	1.25(72)
All (black)	0.83(126)	0.82(186)	0.9(191)	1.22(191)	1.61(191)	1.44(191)	1.28(186)	1.09(126)



Anti-S	13.8–36.7	13.8–43.2	28.2–53.9	35.4–67	41.8–84.2	51.7–125	65.2–4705	81.5–4705
IL6	1	2	3	4	5	6	7	8
<3.24 (red)	0.32(26)	0.3(43)	0.41(50)	0.68(57)	1.07(51)	1.73(41)	1.5(36)	1.56(22)
<7.02 (blue)	0.48(73)	0.62(104)	0.62(102)	0.83(100)	0.92(92)	1.18(91)	0.89(81)	0.71(54)
>7.02 (green)	1.13(53)	1.41(82)	1.43(89)	1.2(91)	1.23(99)	1.13(100)	1.29(105)	1.21(72)
All (black)	0.76(126)	0.86(186)	0.85(191)	1.02(191)	1.06(191)	1.14(191)	1.04(186)	0.91(126)

Figure 3.
OS (A) and PFS (B) depicted by STEPP. Eight overlapping subgroups of patients with different baseline anti-SEA/E-120 (anti-S; pmol/mL) and subsets of patients with different baseline IL6 (pg/mL) are shown.

Cytokine production. In response to Nap-induced T-lymphocyte activation, enhanced plasma concentrations of cytokines are seen. Nap caused an increase of cytokines (peaking 2 to 3 hours post bolus) including IL2, IL6, IL10, IFNγ, and TNFα (Supplementary Fig. S4). The induced systemic cytokine levels were though negligible during cycles 2 and 3. The different cytokines had distinct plasma–time profiles and showed increased concentrations in SG. Furthermore, the cytokine (e.g., IL2) production was most

Table 3. Treatment-emergent adverse events of the ITT and SG populations. Adverse events more common in Nap-treated patients are tabulated; *n* (%)

	ITT				SG			
	Nap + IFNα ($n = 260$)		IFNα ($n = 259$)		Nap + IFNα ($n = 67$)		IFNα ($n = 63$)	
Adverse events	All grades	≥Grade 3[a]	All grades	≥Grade 3[a]	All grades	≥Grade 3[a]	All grades	≥Grade 3[a]
Pyrexia	154 (61%)	6 (2%)	111 (43%)	1 (0%)	45 (67%)	3 (4%)	25 (40%)	
Vomiting	94 (37%)	2 (1%)	21 (8%)	3 (1%)	31 (46%)		3 (5%)	1 (2%)
Nausea	88 (35%)	3 (1%)	30 (12%)	2 (1%)	27 (40%)		6 (10%)	
Chills	69 (27%)	6 (2%)	26 (10%)		22 (33%)	2 (3%)	6 (10%)	
Diarrhea	50 (20%)	3 (1%)	12 (5%)	2 (1%)	15 (22%)	2 (3%)	6 (10%)	1 (2%)
Back pain	48 (19%)	13 (5%)	16 (6%)	2 (1%)	14 (21%)	2 (3%)	5 (8%)	1 (2%)
Hypotension	28 (11%)	4 (2%)	2 (1%)		10 (15%)	1 (1%)		
Tachycardia	17 (7%)		1 (0%)		6 (9%)			
Hypertension	16 (6%)	1 (0%)	4 (2%)		3 (4%)		2 (3%)	
Hyperthermia	16 (6%)	2 (1%)	10 (4%)		1 (1%)		5 (8%)	
Hypersensitivity	13 (5%)	7 (3%)	1 (0%)		3 (4%)	2 (3%)		

[a]No grade 4 or 5 toxicities were observed in those adverse events.

pronounced in the patients with low anti-SEA/E-120 (Supplementary Fig. S5; trend analysis with identical overlapping patient subsets also used for Nap exposure, OS, and PFS).

Safety. The majority of the adverse events resulting from treatment with Nap relates to increased levels of cytokines and is expected as a part of the mechanism of action. Pyrexia, vomiting, nausea, chills, and back pain were more common after Nap (Table 3). Those adverse events were often mild and no grade 4 or 5 toxicities were observed. They were transient and seen only during the weeks in association with the Nap treatment cycles. Furthermore, except for back pain no adverse events were detected that could be attributed to immune complex formation in patients with increased anti-SEA/E-120. In conclusion, Nap was well tolerated and had similar safety profile in the ITT population as in SG.

Discussion

Although this study did not meet primary endpoint, addition of Nap to IFN improves OS (HR, 0.59; $P = 0.02$) and PFS (HR, 0.62; $P = 0.02$) in a *post hoc* analysis subgroup of patients with normal plasma levels of anti-SEA/E-120 and low IL6.

The super antigen moiety of Nap has been engineered to have low binding to baseline human antibodies (10). Despite this fact, increased anti-SEA/E-120 antibody levels may affect drug activity and antitumor efficacy. In this study, the baseline concentration of anti-SEA/E-120 antibodies was unexpectedly higher in certain territories and exposure of Nap accordingly low. Most patients in the phase I studies were from the United States, the United Kingdom, and Scandinavia having low anti-SEA/E-120 antibody titers (11), and as expected, most patients in this study from the United Kingdom had also low concentration of baseline anti-SEA/E-120. Certain patients may have elevated levels of anti-SEA/E-120 due to cross-reactivity to previously encountered wild-type Staphylococcus enterotoxins, for example, through infections from *S. aureus*. The geographical variability regarding antibodies directed to SEA/E-120 might be dependent on different patterns of infection and exposure or different genetic background relating to induction preference of antibodies also binding to SEA/E-120. Furthermore, in the previous phase I studies, a substantial number of patients showed no or low increase of anti-SEA/E-120 titers after Nap treatment. It might be speculated that the combination with IFN enhanced the humoral immune response to Nap as essentially all patients in this study had high titers of anti-SEA/E-120 antibodies after the first Nap treatment cycle. The cytokine response patterns with practically no cytokine production after Nap in retreatment cycles indicated that Nap was neutralized by the antibodies and alternative treatment combinations are needed to reach acceptable immune stimulation in the second and further Nap cycles. Timely combinations with treatments applicable in cancer that interfere with antibody production would be beneficial to avoid Nap neutralization. Anti-CD20 mAb treatment as well as treatment with cytostatic drugs interfering with B-cell activation and response like docetaxel used in combination with Nap are possible such options (11). Anti-SEA/E-120 levels were compared after the first treatment cycle in the phase I studies and showed that docetaxel appeared to reduce anti-SEA/E-120 antibody production when it was given one day after the last injection in a treatment cycle with Nap (11).

IL6 is a pleiotropic cytokine (16–18). Healthy individuals have low and rather stable systemic daytime levels of IL6 although that there are some diurnal variation (19–21). The systemic IL6 concentration may increase as a result from many different insults activating inflammation including chronic inflammatory diseases like rheumatoid arthritis (16). IL6 is also implicated in the pathophysiology of various solid tumors (17) and high IL6 levels are prognostic and correlate with tumor metastasis, disease stage, and short survival in several cancers including RCC (15). The IL6/Janus kinase (JAK)/STAT3 pathway is one of the most important signalling pathways associated with tumor development and induction of tumor-induced immune suppression (22). IL6 is therefore a biomarker for immune status of the tumor microenvironment and for a patient's chronic inflammation/immune suppression status in general. At normal and low levels of IL6, disease has not yet tipped the immune status into suppression. Accordingly, patients with nonelevated systemic IL6 levels would be expected to have the best chance of responding to immunotherapy like Nap. Indeed, baseline plasma IL6 was predictive of benefit with tumor vaccine (23, 24) but also for pazopanib (25). In our study, IL6 was shown to be both prognostic for patients with RCC as well as predictive for Nap activity as the subgroup and the trend analysis clearly supports that low baseline anti-SEA/E-120 and IL6 plasma levels independently predict antitumor efficacy after Nap + IFN treatment.

Nap is typically used in cycles of four to five once-daily intravenous injections. In the first phase of a cycle, the T lymphocytes are activated and differentiated into effector cells, which later in the cycle localize to the tumor and mediate their antitumor functions. This treatment schedule can be repeated and is easily combined with other anticancer drug modalities. Present established treatments of RCC include the TKIs and the mTOR inhibitors, and the checkpoint modulators will probably soon also be a part of the armory (2, 4, 5). The results of our study indicate that patients with less advanced metastatic RCC having nonelevated systemic IL6 levels would be expected to have the best chance of responding to immunotherapy like Nap. Therefore, first- or possibly second-line treatments should preferably be combined with Nap treatment. Some patients in our study received treatment with TKIs after end of study (EOS). Comparing OS HRs of Nap effects in SG patients treated with any TKI after EOS with patients not receiving a TKI after EOS showed very similar results indicating that the Nap OS effects were compatible with additional treatment with a TKI. Treatments with TKIs like sunitinib, pazopanib, and axitinib (2) would easily be initiated with a Nap treatment cycle and additional Nap cycles could be given during, for example, sunitinib vacation weeks (26). It should not be excluded though that Nap cycles should be given during continuous exposure with TKIs as it has been demonstrated that TKIs affect immune cell subpopulations including Tregs, myeloid-derived suppressor cells, as well as T and NK cells (27). Addressing immunosuppressive cells might improve the antitumor-directed T-cell activity, thereby providing a rational for the combination of TKIs like sunitinib with Nap. Furthermore, addition of checkpoint modulators, for example, nivolumab or pembrolizumab, to Nap treatment would potentially both contribute to the Nap effects by blocking PD-1 pathway associated immune suppression at baseline but also interfere with the Nap-induced tumor-infiltrating T-lymphocyte IFNγ upregulation of PD-L1 on tumor cells and tumor associated myeloid cells (6).

There is a tendency for worse OS and PFS after Nap treatment in the *post hoc* analyses patient subgroups with high IL6 or anti-SEA/E-120. The inflammatory biomarker IL6 might indicate that the patients with high cytokine levels have already tipped their immune balance into immune suppression and activation by Nap would hypothetically stimulate and increase immune suppression and tumor growth. In the case with increased anti-SEA/E-120 antibody levels at the initiation of Nap treatment, immune complex formation might corrupt the beneficial immune stimulation, block tumor targeting of Nap, and result in immunosuppressive signals instead. The baseline biomarkers IL6 and anti-SEA/E-120 are therefore not only important to select patients that benefit from Nap treatment but also instrumental to exclude patients responding inadequately to Nap.

Although the study did not meet primary endpoint, addition of Nap to IFN might improve OS and PFS in a subgroup of patients with low IL6 and normal levels of anti-SEA/E-120. Addition of Nap to IFN result in predicted transient immune-related AEs and the treatment has an acceptable safety profile. The results warrant further studies with Nap alone, in combination with established (e.g., sunitinib, axitinib) or experimental (anti-CTLA-4, anti-PD1) treatments in the baseline defined RCC subgroup. More generally, as baseline IL6 appears to be prognostic and predictive of outcome on treatment with TKIs and immunotherapies, this may be a stratification factor for RCC studies.

Disclosure of Potential Conflicts of Interest

R.E. Hawkins reports receiving commercial research grants from GlaxoSmithKline, Novartis, and Pfizer; speakers bureau honoraria from Bristol-Meyers Squibb, GlaxoSmithKline, Novartis, and Pfizer; and is a consultant/advisory board member for Pfizer. G. Hedlund, G. Forsberg, and O. Nordle have ownership interest (including patents) in Active Biotech. T. Eisen is an employee of AstraZeneca; reports receiving commercial research grants from Bayer, GlaxoSmithKline, and Pfizer and other research grants from AstraZeneca; has ownership interest (including patents) in AstraZeneca; and is a consultant/advisory board member for Aveo, Bayer, Bristol-Meyers Squibb, GlaxoSmithKline, Immatics, Novartis, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: R.E. Hawkins, T. Ganev, G. Hedlund, G. Forsberg, O. Nordle, T. Eisen

Development of methodology: R.E. Hawkins, T. Ganev, G. Hedlund, O. Nordle

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.E. Hawkins, M. Gore, M. Harza, O. Karyakin, Y. Shparyk, V. Bondar, O. Gladkov, T. Ganev, S. Polenkov, I. Bondarenko, T. Eisen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.E. Hawkins, T. Ganev, R. Khasanov, G. Hedlund, G. Forsberg, O. Nordle, T. Eisen

Writing, review, and/or revision of the manuscript: R.E. Hawkins, M. Gore, Y. Shparyk, V. Bondar, O. Gladkov, M. Harza, O. Karyakin, T. Ganev, I. Bondarenko, G. Hedlund, G. Forsberg, O. Nordle, T. Eisen

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Bondar, T. Ganev, G. Hedlund, O. Nordle

Study supervision: R.E. Hawkins, T. Ganev, P. Karlov, G. Hedlund

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References

1. Abe H, Kamai T. Recent advances in the treatment of metastatic renal cell carcinoma. Int J Urol 2013;20:944–55.
2. Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, et al. EAU guidelines on renal cell carcinoma: 2014 update. Eur Urol 2015;67:913–24.
3. McDermott DF, Regan MM, Clark JI, Flaherty LE, Weiss GR, Logan TF, et al. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J Clin Oncol 2005; 23:133–41.
4. McDermott DF, Drake CG, Sznol M, Choueiri TK, Powderly JD, Smith DC, et al. Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab. J Clin Oncol 2015;33:2013–20.

5. Gangadhar TC, Salama AK. Clinical applications of PD-1-based therapy: a focus on pembrolizumab (MK-3475) in the management of melanoma and other tumor types. OncoTargets Ther 2015;8:929–37.
6. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 2015; 27:450–61.
7. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol 2015;33:1974–82.
8. Galluzzi L, Vacchelli E, Bravo-San Pedro JM, Buque A, Senovilla L, Baracco EE, et al. Classification of current anticancer immunotherapies. Oncotarget 2014;5:12472–508.
9. Hedlund G, Eriksson H, Sundstedt A, Forsberg G, Jakobsen BK, Pumphrey N, et al. The tumor targeted superantigen ABR-217620 selectively engages TRBV7–9 and exploits TCR-pMHC affinity mimicry in mediating T cell cytotoxicity. PLoS One 2013;8:e79082.
10. Forsberg G, Skartved NJ, Wallen-Ohman M, Nyhlen HC, Behm K, Hedlund G, et al. Naptumomabestafenatox, an engineered antibody-superantigen fusion protein with low toxicity and reduced antigenicity. J Immunother 2010;33:492–9.
11. Borghaei H, Alpaugh K, Hedlund G, Forsberg G, Langer C, Rogatko A, et al. Phase I dose escalation, pharmacokinetic and pharmacodynamic study of naptumomabestafenatox alone in patients with advanced cancer and with docetaxel in patients with advanced non-small-cell lung cancer. J Clin Oncol 2009;27:4116–23.
12. Eisen T, Hedlund G, Forsberg G, Hawkins R. Naptumomab estafenatox: targeted immunotherapy with a novel immunotoxin. Curr Oncol Rep 2014;16:370.
13. Elkord E, Burt DJ, Sundstedt A, Nordle O, Hedlund G, Hawkins RE. Immunological response and overall survival in a subset of advanced renal cell carcinoma patients from a randomized phase 2/3 study of naptumomabestafenatox plus IFN-alpha versus IFN-alpha. Oncotarget 2015;6:4428–39.
14. Lazar AA, Cole BF, Bonetti M, Gelber RD. Evaluation of treatment-effect heterogeneity using biomarkers measured on a continuous scale: subpopulation treatment effect pattern plot. J Clin Oncol 2010;28: 4539–44.
15. Negrier S, Perol D, Menetrier-Caux C, Escudier B, Pallardy M, Ravaud A, et al. Interleukin-6, interleukin-10, and vascular endothelial growth factor in metastatic renal cell carcinoma: prognostic value of interleukin-6–from the GroupeFrancaisd'Immunotherapie. J Clin Oncol 2004; 22:2371–8.
16. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspect Biol 2014;6:a016295.
17. Rossi JF, Lu ZY, Jourdan M, Klein B. Interleukin-6 as a therapeutic target. Clin Cancer Res 2015;21:1248–57.
18. Schaper F, Rose-John S. Interleukin-6: Biology, signaling and strategies of blockade. Cytokine Growth Factor Rev 2015 Oct;26(5):475–87.
19. Marques-Vidal P, Bochud M, Bastardot F, Luscher T, Ferrero F, Gaspoz JM, et al. Levels and determinants of inflammatory biomarkers in a Swiss population-based sample (CoLaus study). PLoS One 2011;6:e21002.
20. Todd J, Simpson P, Estis J, Torres V, Wub AH. Reference range and short- and long-term biological variation of interleukin (IL)-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. Cytokine 2013;64:660–5.
21. Straub RH, Cutolo M. Circadian rhythms in rheumatoid arthritis: implications for pathophysiology and therapeutic management. Arthritis Rheum 2007;56:399–408.
22. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009;9:798–809.
23. Harrop R, Treasure P, de Belin J, Kelleher M, Bolton G, Naylor S, et al. Analysis of pre-treatment markers predictive of treatment benefit for the therapeutic cancer vaccine MVA-5T4 (TroVax). Cancer Immunol Immunother 2012;61:2283–94.
24. Quoix E, Ramlau R, Westeel V, Papai Z, Madroszyk A, Riviere A, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. Lancet Oncol 2011;12:1125–33.
25. Tran HT, Liu Y, Zurita AJ, Lin Y, Baker-Neblett KL, Martin AM, et al. Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials. Lancet Oncol 2012;13:827–37.
26. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, et al. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med 2013; 369:722–31.
27. Seliger B, Massa C, Rini B, Ko J, Finke J. Antitumour and immune-adjuvant activities of protein-tyrosine kinase inhibitors. Trends Mol Med 2010; 16:184–92.